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Patent Application of

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for

**TITLE OF THE INVENTION**

Compositions and Methods for Treating Lung Cancers and COPD

**CROSS REFERENCE TO RELATED APPLICATIONS**

This application is a CIP of application No. 09/132,153 filed 08/11/98, abandoned.

The present application teaches the novel approach of reversing direction of administration of therapeutic over prior art (i.e. going from tissue side toward the circulatory system versus prior art's approach of going from the circulatory system outwards toward tissue) combined with novel use of dosaging to achieve inhibitory, cytotoxic, or toxic affects versus prior arts cytotoxic uses.

As such, present application is related to several other applications filed by applicant.

Application 09/699,913 for locally injectable chemotherapeutics filed 10/30/00 and issued as patent 6,342,520 on 1/29/02 employed cytotoxic doses, the reversed administration method, and adjusted administration intervals to provide a locally injectable chemotherapeutic treatment regimen without systemic toxicity. In one embodiment, present invention uses reversed administration by inhalation and cytotoxic doses to provide a chemotherapeutic treatment regimen for neoplasms in the lungs, without inducing systemic toxicity.

In one embodiment of present invention, inhaled trichothecenes, at inhibitory doses, are used to indefinitely hold the cancer in check by dismantling the cancer's cell cycle control system. In application 09/965,483 filed 9/26/01 and issued as patent 6,346,251 on 2/12/02 for treating psoriasis applicant used reversed administration by topical application and inhibitory doses to dismantle the cell cycle control system of both psoriatic and endothelial cells as two of several pathways inhibited as a treatment for psoriasis.

In one embodiment of present invention, inhaled trichothecenes, at inhibitory doses, are used in the interim periods between administrations of prior art chemotherapeutics to prevent cancer cells from progressing through the cell cycle. In application 09/991,427 filed 11/16/01 for cancer accelerants and phase synchronization methods, applicant discloses why prior art phase specific regimens fail to achieve appreciable cure rates. The failure relates to Gompertzian acceleration, or reduction in cancer cell cycle time, induced by the chemotherapeutic reduction in tumor size, which in turn results in the cancer's lack of phase synchronicity to subsequent administrations of the phase specific chemotherapeutic(s). In application 09/991,427 applicant uses endocrine blockers to conform endocrine dependent cancers to a given chemotherapeutic regimen (i.e. keep the susceptible phase in the cancer synchronized to subsequent administrations of the phase specific chemotherapeutic). In present invention applicant uses inhibitory doses of trichothecenes to conform cancers in the lung to a given chemotherapeutic administration regimen.

In one embodiment of present invention, inhaled trichothecenes, at toxic doses, are used to kill off a desired percentage of lung tissue. In application 09/965,198 filed 9/26/01 and issued as patent 6,355,251 on 3/12/02 for epidermal chemexfoliation applicant uses reversed administration by topical application and toxic doses to kill off a desired percentage of epidermal cells.

In present invention applicant teaches use of trichothecenes for treatment of hyperproliferative and hyperactive conditions of indigenous lung tissue. In application 09/664,014 filed 09/19/00 applicant discloses using inhaled trichothecene for viral lung infections.

## STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

Not Applicable.

## INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ON A CD

Not Applicable

## BACKGROUND OF THE INVENTION - FIELD OF THE INVENTION

Prior art's use of trichothecene has been limited to cytotoxic doses for treating cancer and dates back to the early 1980s. Anguidine, a simple trichothecene, was administered in cytotoxic doses, however its use was abandoned after Phase II results showed overall tumor response rate was low and there was considerable hematologic toxicity. U.S. Pat. 4,744,981 and 4,906,452 embody the direction prior art took to solve the systemic toxicity problem caused by trichothecene's lack of specificity in cellular internalization and blood insolubility; they proposed using conjugates of trichothecene with monoclonal or polyclonal antibodies to selectively deliver the toxin to tumors and proposed glycosylation of trichothecenes to increase blood solubility. Applicant took an exactly opposite approach to prior art and demonstrated how certain trichothecenes could be used unconjugated and unglycosylated to treat tumors by reversing the direction of administration from tissue to blood (interstitial perfusion), dispersing the trichothecenes between the intercellular spaces and then using the gap junction transport system to cleanly localize the trichothecene in the tumor, for which applicant was granted U.S. Patent 6,342,520.

In the present invention, applicant uses inhalation to achieve the novel tissue side administration method, without inducing systemic toxicity. Applicant will provide novel administration intervals for using trichothecene at cytotoxic levels to achieve high tumor kill rates. Additionally, applicant has extended the utility of trichothecenes by employing inhibitory dose levels (i.e. lower than cytotoxic doses) as well as toxic dose levels (i.e. higher than cytotoxic doses) to provide novel therapeutic benefits including conforming a cancer to a given chemotherapeutic regimen, indefinitely holding a cancer in check, providing a method of chemical debridement and reducing inflammatory responses .

## BACKGROUND OF THE INVENTION - DESCRIPTION OF RELATED ART

There are no chemotherapeutics in use today that function by broad spectrum protein synthesis inhibition. Most chemotherapeutic agents in use today are cell cycle active; that is, they are cytotoxic mainly to actively cycling cells. Alkylating agents are among the most widely used anti tumor agents and are efficient at cross-linking DNA, leading to strand breakage. Alkylating agents include cyclophosphamide, ifosfamide, melphalan, busulfan, mechlorethamine (nitrogen mustard), chlorambucil, thiotepa, carmustine, lomustine as well as platinum compounds such as cisplatin and carboplatin, which are not true alkylating agents also lead to covalent cross linking of DNA.

Purine/pyrimidine analogs/antimetabolites induce cytotoxicity by serving as false substrates in biochemical pathways. They include cytarabine, fluorouracil, gemcitabine, cladribine, fludarabine, pentostatin, hydroxyurea, and methotrexate. Topoisomerase inhibitors interfere with the enzymes topoisomerase 1 and topoisomerase 2, responsible for mediating conformational and topological changes in the DNA required during transcription and replication. These agents include daunorubicin, doxorubicin, idarubicin, etoposide, teniposide, dactinomycin, and mitoxantrone. Plant Alkaloids include vincristine, vinblastine, and vinorelbine which inhibit microtubule assembly by binding to tubulin and docetaxel and paclitaxel which function by stabilizing microtubules and preventing their disassembly. Antitumor Antibiotics include bleomycin that induces DNA strand breakage through free radical generation and Mitomycin C which cross links DNA. Other Agents include dacarbazine and procarbazine which act as alkylating agents to damage DNA and L-Asparaginase, the only enzyme used as a anti tumor agent, which acts by depletion of extracellular pools of asparagine.

### Principles of Chemotherapeutic Administration.

Chemotherapeutic agents exhibit a dose response effect. At sufficiently low concentrations no cytotoxicity is observed. At increasing concentrations, cell kill is proportional to drug exposure. At high concentrations, the effect reaches a plateau. Drugs that are cell cycle active, but not phase specific, characteristically have steep dose response curves: An increase in the drug concentration by an order of magnitude or more results in a proportional increase in tumor cell kill. By contrast, the dose response curve of phase specific agents typically is linear over only a narrow range. These agents are less suitable for dose escalation and increased tumor cell kill is observed after

prolonged exposure as a larger percentage of the tumor cells enter the cell cycle.

Chemotherapy employs two principles in administration: Therapeutic Index Dosaging and Cyclical Administration (HPIM 527 -528 Pharmacodynamics section).

The therapeutic index represents the difference between the response of the tumor and response of normal tissue for a given dose of chemotherapeutic. Normal cells are also susceptible to the cytotoxic effects of chemotherapeutic drugs and exhibit a dose-response effect, but the response curve is shifted relative to that of malignant cells (see HPIM P. 528, figure 86-3). This difference represents the therapeutic index. The toxicity to normal tissue that limits further dose escalation is the “dose-limiting toxicity”. The dose just below this point is the “maximum tolerated dose”. Proliferative normal tissues such as the bone marrow and gastrointestinal mucosa are generally the most susceptible to chemotherapy-induced toxicity. The usefulness of many chemotherapeutics is limited by the fact that they have a narrow therapeutic index.

Cyclical administration is used to allow normal rapidly proliferating tissue such as hemopoietic stem cells to recover and blood counts to reach their normal levels. Most chemotherapeutics are administered in cycles of 21 to 28 days and 6 to 8 cycles are typically used. The number of administration cycles required to completely eradicate a tumor is dependent on the tumor kill rate of the therapeutic the number of cycles required is determined using the Skipper log cell kill model. To completely eradicate a tumor it is necessary to get below the mathematical 1 surviving cell number. As an example, to kill a 10 billion cell tumor with a chemotherapeutic that kills 95% of the tumor cells each administration cycle (5% survive) would require 8 cycles of chemotherapy (i.e.  $10,000,000,000 \times .05 \times .05 \times .05 \times .05 \times .05 \times .05 \times .05 \times .05 = .39$ ). However, this assumes the tumor does not grow in the period when the chemotherapeutic is not administered (“off” period).

## BRIEF SUMMARY OF THE INVENTION

The current invention proposes administration, by inhalation, of locally cytotoxic doses of certain sesquiterpene epoxides (trichothecenes) reduce viability of neoplasms in the lungs. The current

invention proposes administration, by inhalation, of locally inhibitory doses of certain sesquiterpene epoxides (trichothecenes) to inhibit proliferation of neoplasms in the lungs. Methods are also provided for chemical debridement of diseased cells in the lungs of COPD patients using locally toxic doses as well as inhibition of inflammatory responses in the lungs by inhalation of locally inhibitory doses of trichothecenes.

The present invention both overcomes the inability of prior art to use PSIs as chemotherapeutics and present invention also provides several other novel therapeutic treatment methods not envisioned or anticipated under prior art.

First, present invention will provide methods for using PSIs as chemotherapeutics using locally cytotoxic doses. It will be shown how certain trichothecenes can be used to provide superior chemotherapy for treatment of cancers in the lung, with a further advantage of achieving specificity to the cancer tissues in the lungs without toxicity to normal lung tissue and without systemic toxicity. This is a goal that has eluded prior art.

Second, present invention will provide novel and unobvious methods for indefinitely inhibiting growth of cancers and cancer related angiogenesis in the lungs through locally inhibitory doses of therapeutics of present invention.

Third, present invention will provide novel and unobvious methods of enhancing existing chemotherapeutic treatments of cancers in the lungs by using locally inhibitory doses to 1) insure the integrity of the Skipper log cell kill model, 2) to prevent chemotherapeutic induced Gompertzian acceleration of tumor growth rates and the associated low cure rates resulting from the loss of cancer's synchronicity to phase specific chemotherapeutics, and 3) to reduce the ability of multi drug resistant cancers to fight off chemotherapeutics.

Fourth, present invention will provide novel methods of treating patients suffering from Chronic Obstructive Pulmonary Disease (COPD) using locally toxic dose levels. Present invention will provide methods of chemical debridement of malfunctioning lung cells in COPD patients.

Fifth, using inhibitory dose levels, present invention will provide novel methods of inhibiting inflammation in the lungs by inhibiting or preventing synthesis of protein mediators of anaphylaxis.

#### BRIEF DESCRIPTION OF DRAWING FIGURES

FIG. 1A shows the hyperactive protein synthesis inhibiting dose profile in human cells of Roridin A, a representative macrocyclic trichothecene.

FIG. 1B shows the hyperactive protein synthesis inhibiting dose profile in human cells of Satratoxin G, a representative macrocyclic trichothecene.

FIG. 2A shows the hyperactive protein synthesis inhibiting dose profile in human cells of T - 2, a representative simple trichothecene.

FIG. 2B shows the hyperactive protein synthesis inhibiting dose profile in human cells of DAS, a representative simple trichothecene.

FIG. 3 shows the hyperactive Cyclin/CDK protein synthesis activity required at various points in the cell cycle to drive the cell through the cell cycle (i.e. the cell cycle control system).

FIG. 4 shows the molecular pathways depending on the hyperactive protein synthesis of the G1 cyclin/CDK proteins required to drive the cell through the G1 phase of the cell cycle.

FIG. 5A shows a cross section of the spacing between two adjacent cell as well as the related structures referred to as gap junctions that connect adjoining cells.

FIG. 5B shows the particle size limitation for gap junction transport between two adjacent cells.

## DETAILED DESCRIPTION OF THE INVENTION

The treatments disclosed below involve administration of biologically active trichothecenes by inhalation to kill cancer cell populations in the lungs, to inhibit the growth of cancer and endothelial cell populations in the lungs, to destroy diseased tissue in the lungs, and to inhibit production of mediators of anaphylaxis. Materials and methods for achieving this are described below.

### Overview of Protein Synthesis Inhibition:

The most fundamental function a cell is protein synthesis (i.e. expression of its DNA). Proteins make up ~ 60% of a dry cell's mass by weight. In very broad and general terms, as cells mature and differentiate in the body, they reach an equilibrium in protein synthesis and protein degradation and settle down to perform their given function in this relative state of homeostasis. There are two notable exceptions that cause massive perturbations to this homeostasis: 1) when a cell is called upon to grow and divide and 2) when certain secretory cells are called upon to produce large amounts of proteins for secretion. Although the cell signaling pathways, intracellular transduction pathways, and spectrum of protein(s) to be produced are quite different in growth versus secretion, normal growth and secretion events share one major similarity in their end result: massively accelerated protein synthesis. A cell that is called on to grow (cycling cell) has as much as 5 times the protein synthesis activity of a non cycling cell. Likewise, secretory cells such as those of the immune system become protein factories producing massive amounts of antibodies, mediators, growth factors, or other proteins when stimulated to do so.

There are also abnormal conditions such as cancer and viral infections that share the same property of hyperactive protein synthesis versus normal quiescent cells. Cancer is a growth and divide type event, and even though the signaling mechanism is different in that it is self-induced intracellularly by several genetic mutations, the end result is also hyperaccelerated protein synthesis characteristic of a cycling cell. Viruses invade a cell, parasitize the host cellular machinery, and convert the cell into a factory producing massive amounts of viral proteins, much like a secretory cell.

Inhibiting protein synthesis affects cells in a dose dependent manner and affects actively cycling cells differently than non cycling cells. At low doses, protein synthesis inhibitors (PSIs) stop



actively cycling cells from cycling without killing them by preventing the hyperactive Cyclin/CDK protein synthesis activity required to drive the cell cycle control system as shown in FIG. 3 (hereinafter referred to as inhibitory or G zero inducing dose). Inhibitory doses also stop hyperaccelerated protein synthesis by secretory cells.

At moderate doses PSIs exhibit toxicity to actively cycling cells (hereinafter referred to as the cytotoxic dose). Interference with protein synthesis has lethal consequences for actively cycling cells in various phases of the cell cycle. Actively cycling cells have as much as 500% more protein synthesis activity than non cycling cells. Several pathways are likely involved with the observed cytotoxicity of protein synthesis inhibitors to actively cycling cells. Temporary disruption of spindle microtubules preferentially kills many abnormally dividing cells. Microtubules are highly labile structures and are highly sensitive to concentrations of the protein tubulin for their rapid elongation and contraction. Interfering, even slightly, with the concentrations of free tubulin by a protein synthesis inhibitor, would prevent the critical concentration required for polymerization (rapid elongation) and formation of the mitotic spindle required for cell division. Reducing tubulin concentrations at an inappropriate time in the cell's division cycle would cause premature depolymerization (rapid shrinking) of the microtubules at an inappropriate time which would literally result in portions of the DNA being torn apart. Prior art chemotherapeutics that work by disruption of spindle microtubules to kill cancer include vinblastine, vincristine, paclitaxel and docetaxel. Vincristine and vinblastine inhibit microtubule assembly by binding to tubulin and thus are cytotoxic predominantly during the M phase of the cell cycle. Paclitaxel and docetaxel stabilize microtubules preventing their disassembly.

Several key proteins need to be switched on at a high rate of synthesis at various stages of the cell cycle. Histones, which are required for formation of new chromatin, are made at a high rate only in S phase and the same is true for some enzymes that manufacture deoxyribonucleotides and replicate DNA. In G1, large amounts of protein synthesis is required to grow the cell to nearly twice its size. Compositions of present invention interfere with all of these processes.

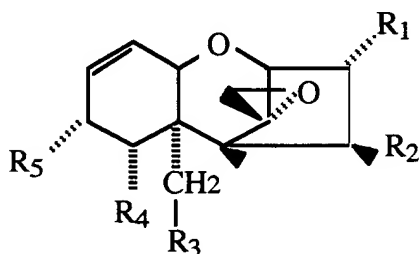
At high doses, PSIs exhibit toxicity to all cells ( hereinafter referred to as the toxic dose).

### Trichothecenes Defined:

Fungi of the genera *Fusarium*, *Myrothecium*, *Trichoderma*, *Stachybotrys* and others produce Trichothecene mycotoxins. Trichothecenes constitute a family of fungal sesquiterpene epoxides that inhibit protein synthesis. Trichothecene mycotoxins are low molecular weight (250-700 daltons), non volatile compounds, and of over 150 trichothecenes have been identified. There are two broad classes: those that have only a central sesquiterpenoid structure and those that have an additional macrocyclic ring (simple and macrocyclic trichothecenes, respectively).

As used in this application, “therapeutics”, “biologically active agent”, or “trichothecene” are defined as either simple or macrocyclic trichothecenes and include molecules of the following general chemical formulas: Simple trichothecenes are categorized into three groups with the following chemical formulas:

#### Group A:



Wherein  $R_1$  is H, OH, or  $O-C(=O)-CH_3$ ;

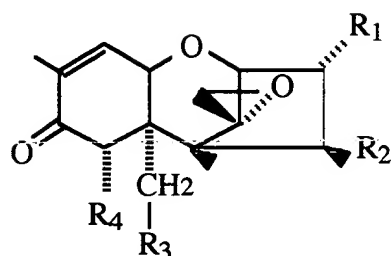
$R_2$  is H, OH, or  $O-C(=O)-CH_3$ ;

$R_3$  is H, OH, or  $O-C(=O)-CH_3$ ;

$R_4$  is H or OH; and

$R_5$  is H, OH,  $O-C(=O)-CH_3$  or  $C(=O)-O-CH_2(CH_3)_2$ .

Group B:



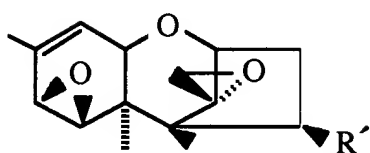
Wherein  $R_1$  is H, OH, or  $O-\overset{\overset{O}{\parallel}}{C}-CH_3$ ;

$R_2$  is H, OH,  $O-\overset{\overset{O}{\parallel}}{C}-CH_3$  or  $O-\overset{\overset{O}{\parallel}}{C}-CH=CH-CH_3$ ;

$R_3$  is H, OH, or  $O-\overset{\overset{O}{\parallel}}{C}-CH_3$ ;

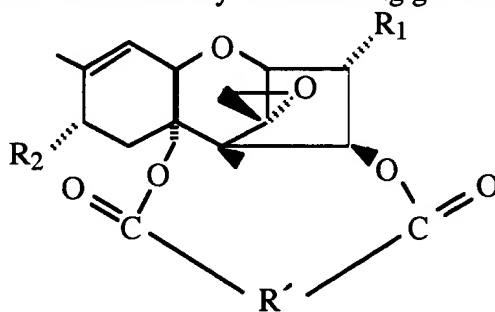
$R_4$  is H, OH, or  $O-\overset{\overset{O}{\parallel}}{C}-CH_3$ ;

Group C:



Wherein  $R'$  is OH or  $O-\overset{\overset{O}{\parallel}}{C}-CH=CH-CH_3$ .

Macrocyclic Trichothecenes can be described by the following general chemical formulas:



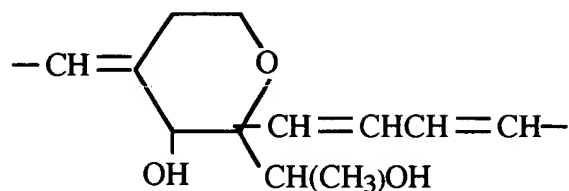
Wherein  $R_1$  is OH, or  $OC-\overset{\overset{O}{\parallel}}{C}-CH_3$ ;

$R_2$  is H, OH,  $O-\overset{\overset{O}{\parallel}}{C}-CH_3$  or  $OCOCH_2CH(CH_3)_2$ ; and

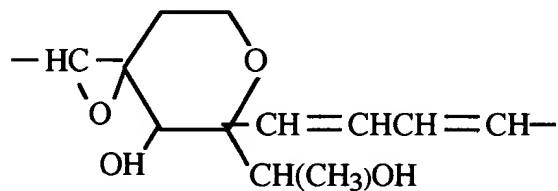
$R'$  is any molecule composed predominantly, or in its entirety, of combinations of C, H, and O:

Some representative examples of R' include:

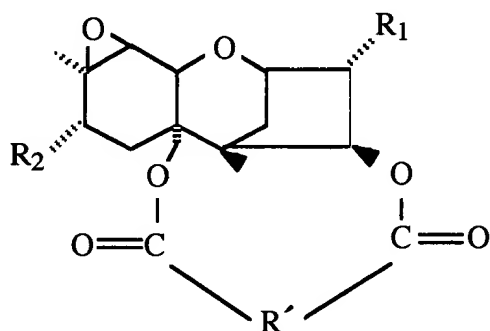
Satratoxin H:



Satratoxin G:



or molecules of the following general formula:



Wherein R<sub>1</sub> is H, OH, or  $\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-\text{CH}_3$ ;

R<sub>2</sub> is H, OH,  $\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-\text{CH}_3$  or  $\text{OCOCH}_2\text{CH}(\text{CH}_3)_2$ ; and

R' is any molecule composed predominantly, or in its entirety, of combinations of C, H, and O.

A more comprehensive listing of trichothecenes is included in U.S. Patents 4,744,981 and 4,906,452, incorporated herein by reference.

Trichothecenes are fast acting potent inhibitors of protein synthesis in eucaryotic cells. Their main effects are on rapidly proliferating tissues such as bone marrow, skin, mucosa epithelia, and germ

cells. The sesquiterpenoid ring binds to ribosomes, inhibiting protein synthesis. The macrocyclic ring enhances cell binding and internalization.

Trichothecenes are invisible to the immune system since they neither contain nor produce amino acids. Since trichothecene molecules contain only carbon, hydrogen, and oxygen they are not subject to proteolytic degradation. U.S. Pat. No. 4,906,452 ( column 11 first paragraph) further discloses that some studies of the rates at which certain trichothecenes are converted into biologically inactive molecules (apotrithothecenes) found that macrocyclic trichothecenes are inactivated quite slowly and only by intracellular acid catalysis as might occur in lyzosomes.

Trichothecenes are extremely stable to heat and ultraviolet light inactivation. Heating to 500° F for 30 minutes is required for inactivation. Brief exposure to NaOH destroys toxic activity. These substances are relatively insoluble in water but are highly soluble in ethanol, methanol, and propylene glycol.

#### Internalization and Localization Attributes:

The novelty and unobviousness of applicant's proposal stems in part from several attributes of trichothecene that are not generally known to prior art. Certain trichothecenes are capable of rapid cellular internalization with extreme reluctance to enter the blood stream as well as being capable of gap junction transport and having extremely slow intracellular inactivation times. Cells of an organ or tissue type are spaced 2 - 4 nm apart as shown in FIG. 5A and connexons in the bi-lipid cell membrane connect adjacent cells metabolically by what are referred to as gap junctions. Gap junctions allow molecules smaller than 1000 daltons or less than ~ 1.5 nm to pass as shown in FIG. 5B. Amino acids range between .5 - 1 nm in size and as such individual amino acids are shared between cells but not macromolecules of amino acids.

Trichothecenes are extremely small at around 500 daltons ranging up to 750 daltons ( or ~ .8 nm to ~1.2 nm). As such they are capable of dispersing via in the 2 - 4 nm spaces between cells and once internalized are capable of travel through the 1.5 nm gap junction transport system between cells. Interstitial perfusion by inhalation, particularly of rapidly internalizable and highly blood

insoluble macrocyclic trichothecenes as proposed by applicant, is the first step in homogenous distribution. The second more precise step is accomplished by the gap junctions transport system, distributing the internalized trichothecenes throughout the connected organ or tissue mass, without being transported to unrelated structures or appreciably entering general circulation.

Conjugated trichothecenes are too large for traveling between cells or through gap junctions. Conjugating trichothecenes with monoclonal or polyclonal antibodies greatly increases their size. The basic structural unit of an antibody molecule consists of four polypeptide chains and contains ~ 1320 amino acids. Adding a single average amino acid of ~ .7 nm to a 1 nm trichothecene already exceeds the 1.5 nm gap junction diameter preventing use of the gap junction transport system (and that is without provisions for linker molecules). A second amino acid already exceeds the lower limit of spacing between cells.

#### Preparation of Trichothecenes:

Fungi can be grown in culture and the trichothecenes extracted by centrifugal partition chromatography as described in Tani et. al. and described in other literature such as Onji et. al. (Onji, Y., Aoki, Y., Yamazoe, Y., Dohi, Y., and Moriyamam, T., 1988 *Isolation of nivalenol and fusarenon-X from pressed barley culture by centrifugal partition chromatography, Journal Liquid Chromatography*, 11:2537-2546) or Jarvis et al. (Jarvis, B.B., R.M., Eppley, and E.P. Mazzola, 1983 *Chemistry and Bioproduction of the Macrocyclic Trichothecenes*, p 20-38. In Y. Ueno, *Trichothecenes: chemical, biological, and toxicological aspects*, vol 4. Elsevier Science Publishing Inc., New York) or Sorensen et al. (Sorenson, W.G., Frazer, D. G., Jarvis, B.B., Simpson, J., and Robinson, V.A., *Trichothecene Mycotoxins in Aerosolized Conidia of Stachybotrys atra*, June 1987 *Applied and Environmental Microbiology*, Vol. 53 No. 6, p. 1370-1375) where *S. atra* was grown on sterile rice, autoclaved, dried, and then aerosolized by acoustic vibration and collected on glass-fiber filters and extracted with 90% aqueous methanol.

Alternatively, certain trichothecene mycotoxins can be purchased from companies such as Sigma Chemical Co. St. Louis MO, USA or Wako Pure Chemical Industries, Ltd., Japan, or Wellcome

Research Labs, Buckinghamshire, England or Boehringer-Mannheim, Mannheim, West Germany .

The preferred embodiment of current invention envisions using Satratoxin H as well as other combinations of trichothecenes such as satratoxins G, H, F, roridin E, verrucarins J, and trichoverrols A and B for reasons discussed later. These trichothecenes can be obtained by growing the fungus *stachybotrys atra* on sterile rice and extracting the trichothecenes by centrifugal partition chromatography as described in Tani et. al. or having it grown on sterile rice, autoclaved, dried, and then aerosolized by acoustic vibration and collected on glass-fiber filters and extracted with 90% aqueous methanol as described by Sorensen et al..

#### Method of Administration:

Preferred embodiment of current invention administers trichothecenes by inhalation in their raw dry powder form through commercially available dry powder inhaler devices such as the Pulmicort Turbuhaler breath activated dry powder inhaler (Astra USA Inc., Westborough, MA ) or Galaxo Wellcome's Diskus inhaler. However, any suitable commercially available inhaler devices, nebulizers, or any other suitable means and in combination with any suitable solution or device to facilitate inhalation, retention, or absorption by the lungs may be used. Such devices are commercially available from sources such as Self Care, Emeryville, CA, USA and enclosed examples include the Lumiscope Ultrasonic Nebulizer, the Dura-Neb® 3000 Portable compressor driven nebulizer, the PARI LC plus Nebulizer, the Omron CompAir Compressor Nebulizer System, the SpaceChamber™ aerosol spacer, and other devices.

Alternatively, therapeutics of present invention may also be administered by cigarette (either tobacco or other). Trichothecenes require temperatures of 500° F for 30 minutes for inactivation allowing them to still be biologically active after being inhaled through a lit cigarette. The diseases considered for treatment under present invention such as lung cancer and COPD are primarily caused by smoking. A large number of patients do not quit smoking even after acquiring lung cancer. From a purely medical standpoint, cessation of cigarette smoking does not markedly decrease the cured cancer patient's risk of second malignancy (HPIM p. 501). Methods of mixing additives with tobacco are established in prior art in the tobacco industry. Measurement of

effective dosages of trichothecenes inhaled per cigarette can be determined by using equipment developed in prior art for testing tar and nicotine content inhaled per cigarette or using glass fiber filters and pumps (emulating human inhalation patterns from cigarettes) and measurement of collected dosages could be performed after extraction with 90% aqueous methanol as described in Sorensen et. al. The appropriate number of cigarettes to be smoked to deliver the desired therapeutically effective dosages would then be determined.

#### Dose Determination:

Fig. 1A and 1B show the hyperactive protein synthesis inhibiting dose profile of roridin A and satratoxin G, respectively. Both roridin A and satratoxin G are macrocyclic trichothecenes. By ~ 5 ng/ml both had inhibited almost 100% of the hyperactive protein synthesis. Both did not reduce cell viability at concentrations of 10 ng/ml or less.

Figure 2A and 2B show the hyperactive protein synthesis inhibiting dose profile of T-2 and DAS, respectively. Both T-2 and DAS are simple trichothecenes. By doses of 5 ng/ml both had inhibited ~ 99% of hyperactive protein synthesis. Neither reduced cell viability at concentrations up to 200 ng/ml.

The hyperactive protein synthesis inhibiting profiles were constructed from data collected from in vitro experiments using human epidermoid cells, virally infected with HSV-2 to induce a hyperactive state of protein synthesis, and conducted and reported by Okazaki et. al. in the attached Journal of Agricultural and Biological Chemistry articles.

Conversion of in vitro concentrations to dosages required to achieve in vivo concentrations would be performed by simple mathematical methods. As an example, if a patient has an average lung weight of ~ 1200 grams and one desires to achieve a 5 ng/ml concentration of Satratoxin one would need to administer ~ 5,925 ng of dry satratoxin (i.e. 1 gram = .9875 ml, 1200 gram lungs ~ 1185 ml, 1185 ml X 5 ng/ml = 5,925 ng.) by inhalation methods described above. Adjustments would be made for individual lung size differences and additional tumor mass where applicable.



Inhibitory, Cytotoxic, and Toxic doses are used in various treatment applications discussed later in the application. Using the data from the viral protein synthesis inhibiting model referenced above, dose guidelines for use in the Inhibitory, Cytotoxic, and Toxic embodiments of present invention are listed below in TABLE 1 and TABLE 2. Since no reduction in cell viability was observed at concentrations of less than 10 ng/ml for the two macrocyclic trichothecenes and 200 ng/ml for the two simple trichothecenes, TABLE 3 was constructed assuming two worst case scenarios 1) that none of the inhaled trichothecene is retained by the lungs and instead all of it finds its way to the roughly 42 liters of extracellular water outside the vasculature in the body or 2) more seriously the entire dose is accidentally injected directly into the blood stream which contains roughly 5 liters. TABLE 3 displays the maximum amount of trichothecene, in ng, that would not reduce cell viability systemically under the two scenarios. The maximum locally toxic doses for use in TABLE 2 are taken from TABLE 3.

TABLE 1: Trichothecene in Vitro Concentrations (in ng/ml)

	<u>Inhibitory</u>				<u>Cytotoxic</u>		<u>Toxic</u>
	<u>50%</u>	<u>80%</u>	<u>90%</u>	<u>99%</u>	<u>Min</u>	<u>Max</u>	<u>Lo</u>
Roridin A	1.4	2.0	3.3	5.0	6	10	11
Satratoxin	1.5	2.4	3.9	5.0	6	10	11
T-2	1.6	3.5	4.3	5.0	6	200	201
DAS	2.3	4.0	4.5	5.0	6	200	201

TABLE 2: In Vivo Dose for Average 1200 Gram Lungs ( in ng)

	<u>Inhibitory</u>				<u>Cytotoxic</u>		<u>Toxic</u>	
	<u>50%</u>	<u>80%</u>	<u>90%</u>	<u>99%</u>	<u>Min</u>	<u>Max</u>	<u>Lo</u>	<u>Hi</u>
Roridin A	1659	2370	3911	5925	7110	11850	13035	50000
Satratoxin	1778	2844	4622	5925	7110	11850	13035	50000
T-2	1896	4148	5095	5925	7110	237000	238185	1000000
DAS	2726	4740	5333	5925	7110	237000	238185	1000000

TABLE 3: Worst Case Safe Systemic Levels ( in ng)

	<u>Max. Non Toxic @ 42 Liters</u>	<u>Max. Non Toxic @ 5 Liters</u>
Roridin A	420,000	50,000
Satratoxin	420,000	50,000
T-2	8,400,000	1,000,000
DAS	8,400,000	1,000,000

The above are ballpark numbers for illustrative purposes. The concentrations and doses used should not be construed as “optimal”. As is customary under prior art, all dosages would be further refined and scrutinized by in vivo testing in suitable animal models or in Phase I and II clinical trials on humans as required by the FDA and the lowest concentrations suitable to achieve efficacy would likely be called “optimal”. The “optimal” doses could readily be expected to be much smaller than those presented for use in inhibitory or cytotoxic treatment regimens. Likewise, toxic doses, much higher than those presented above, could likely be more efficacious yet equally safe. Locally toxic doses may also be substituted in place of cytotoxic doses in many instances. The doses presented in this application were done so to fulfill the reduction to practice requirement of this application and are not intended to imply an absolute standard or “optimal” dose but are merely some representative examples of efficacious, yet safe, embodiments of present invention.

#### Alternative Method of Therapeutic Dose Determination:

Although specific dose profiles for therapeutics have been discussed above, present invention can easily be extended to using various other trichothecenes as well as combinations of trichothecenes (to affect the depth of penetration of therapeutics of present invention). Accordingly, a general method for dosage determination of other trichothecenes is provided below.

Human cell lines, including human lung cell lines, are commercially available from several sources including ATCC - American Type Culture Collection, Manassas, VA, USA or ECACC - European Collection of Cell Cultures, Salisbury, Wiltshire, UK or DSMZ - German Collection of Microorganisms & Cell Cultures, Braunschweig, Germany or IZSBS - Istituto Zooprofilattico Sperimentale, Brescia, Italy or ICLC - Interlab Cell Line Collection, Genova, Italy or ECBR -

European Collection for Biomedical Research, Genova, Italy or any other suitable supplier. Human lung cell lines available include both normal and malignant cell lines. As an example, LL24 is a human lung cell line available from ECACC. Examples of malignant cell lines include A-427 human lung carcinoma available from DSMZ and COR-L23 human large cell lung carcinoma available from ECACC, however any other suitable cell line from any other suitable source may be used.

To establish inhibitory, cytotoxic, toxic, and lethal concentrations human lung cell lines (both normal and malignant) would be grown in culture and exposed to various concentrations of trichothecenes by methods described in Okazaki et al. or Tani et al. where human cell lines were grown in Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS). Trichothecenes would be dissolved in dimethyl sulfoxide at a concentration of 20 mg/ml and diluted in Eagle's MEM. Stock solutions (200  $\mu$ g/ml) could be prepared, passed through a 450 - nm Millipore membrane filter and stored at - 20° C until use. Tissue culture plates would be seeded with normal human lung cell lines and other culture plates would be seeded with human lung cancer cell lines. Both sets of cells would then be allowed to proliferate at 37° C until confluent monolayers had formed. The culture plates would then be exposed to various concentrations of the trichothecenes and the number of viable cells periodically counted using trypan blue exclusion after trypsinization. A "Normal Tissue Response" and "Malignant Tissue Response" profile would then be constructed for various trichothecene concentrations under prior art methods as illustrated in HPIM Figure 86-3, pg. 528 as part of constructing a Therapeutic Index profile. Observed recovery time of the malignant cells (below cytotoxic dose levels) would serve as an indicator of intracellular inactivation time for purposes of determining periodicity of administration cycles (versus prior art which uses blood count recovery times to determine periodicity between administration cycles). National Cancer Institutes (NCI) protocols for animal and human testing would subsequently be performed to refine dosage administration.

#### Safety and Efficacy by Inhalation - The Cleveland Infant Model:

The cluster of infant deaths in Cleveland (NIEHS press release and MMWR report) demonstrated, in vivo, in humans, both a usable therapeutic index of certain trichothecenes when administered by

inhalation, as well as their ability to localize in lung tissue without appreciably entering general circulation.

The Cleveland infants served as a model for rapidly proliferating tissues such as cancer. The mean age of the infants was ~ 10 weeks old (range 4 - 16 weeks). At this age, the lungs of infants are growing at an accelerated rate, similar to the accelerated growth rates of cancer. Likewise the effects of inhaled trichothecenes would be analogous to their effect on cancer.

The control group was represented by the adults living in the same household and inhaling the same trichothecenes. The median age of the infant's mothers was 20 years (range 15 - 29 years). At this age, the lungs of adults are not growing at an accelerated rate and are analogous to normal tissue as defined in the context of therapeutic index dose profile determination previously discussed.

Both groups were subjected (inadvertently) to airborne concentrations of trichothecenes produced naturally by the fungus *Stachybotrys atra*. Trichothecenes produced by *S. Atra* include satratoxins H, G, F, roridin E, verrucarins J, and trichoverrols A and B.

The destruction of the rapidly proliferating tissues of the infants was so severe that it resulted in the death of at least 10 infants. No health problems were reported by the adults. This indicated cytotoxic dose levels were inhaled and also demonstrated that a useful therapeutic index exists at which tissue growing at an accelerated rate in the lungs is severely damaged or destroyed and normal tissue is not affected.

This also demonstrated the safety of using trichothecenes by inhalation in adults. Even in the infants, despite the acute pulmonary hemorrhage/hemosiderosis, the inhaled trichothecenes localized in the lungs and did not enter circulation where they would have caused systemic cytotoxicity. Laboratory findings on admission showed a normal white blood cell count (median = 13.8 cells/ cubic mm) in the infants. Red blood cell counts were consistent with the blood loss from the hemosiderosis. No other source of bleeding (i.e. gastrointestinal or nasopharyngeal)

was identified during endoscopic evaluation.

The inhaled trichothecenes are essentially “trapped” between the lumen of the lungs on one side and the circulatory system on the other side, in which they are insoluble. In between this is the lung tissue in which they eventually internalize. The molecular basis for the “localization” of these trichothecenes has been previously disclosed in the “Internalization and Localization Attributes” section of this application. Additionally, the water insolubility of trichothecenes would facilitate their escaping elimination from the lung by the by cilia driven movement of mucus. Cephalad movement of the mucus blanket at .5 - 1 mm/minute normally removes accumulated material from the lungs in ~24 hours. Since environmental tobacco smoke was implicated in the severity of tissue destruction (NIEHS press release) and tobacco smoke is known to inactivate (and eventually destroy) cilia in the lungs, inactivating or destroying cilia results in increased retention of trichothecenes in the lungs, further enhancing both localization and efficacy. Consequently, smokers and smoke damaged lungs, which are the main target of therapeutics of present inventions, should benefit disproportionately over normal healthy lungs. Present invention also envisions deliberately inactivating or destroying cilia prior to administration of trichothecenes to improve localization and efficacy.

Although there have been studies on the rates at which trichothecenes are intracellularly converted into biologically inactive apotrithothecenes, the Cleveland infant model provides a rare glimpse of how slowly macrocyclic trichothecenes are inactivated, in vivo, in the lungs, after inhalation. All infants survived the first hospitalization and were discharged without evidence of hemoptysis after a median length of stay of 10 days, indicating an inactivation time at cytotoxic doses in the ballpark of 10 days.

#### Safety by Inhalation - AMRIID's Aerosolized Administration Model

The safety of using trichothecenes by inhalation can be further substantiated by AMRIID's research on inhalation of aerosolized trichothecenes. The simple trichothecene T-2 was evaluated (see AMRIID Table 2). Even though trichothecenes are some of the most potent toxins by weight, when AMRIID administered T-2 in aerosolized form, T-2 came 25th out of 25 toxins tested for

lethality by inhalation. AMRIID computed the LD50 (lethal dose to 50% of people) by inhalation as 1,210  $\mu\text{g/kg}$  of body weight. This translates to a 84,700,000 ng dose of T-2 being inhaled by a 70 kg person to have a 50% chance of mortality. This contrasts with the 5925 ng maximum inhibitory dose ( $\sim 14,295$  times smaller) for T-2, or the 237,000 maximum cytotoxic dose for T-2 (357 times smaller), or the 1,000,000 maximum toxic dose (85 times smaller) as proposed by present invention in the dose determination section of this application.

### **Cytotoxic Activity**

The cytotoxic mechanisms of action against cancer cells are disclosed by applicant in U.S. Pat. # 6,342,520 for Locally Injectable Chemotherapeutics from column 4 line 29 to the end of column 7, and incorporated herein by reference.

Administration intervals for cytotoxic regimens using trichothecene are different from any prior art administration regimens. Prior art methods administer chemotherapeutics on 7 or 21 day intervals to allow recovery from systemic cytotoxicity, especially hematologic toxicity. Since localized administration of trichothecenes does not result in systemic toxicity, prior art administration intervals have no relevance to present invention. What is relevant for computing the frequency of administration of cytotoxic doses in present invention is the amount of time it takes any surviving cancer cells to inactivate the trichothecenes (i.e. by converting them to apotrithothecenes) plus approximately 8 hours to reassemble their cell cycle control machinery and start cycling again. Methods of computing administration frequency are disclosed by applicant in U.S. patent 6,342,520 from column 13 line 4 to line 53, incorporated herein by reference. Because of the genetic variability in mutation profiles, the preferred method of determining frequency of administration is by using frequent PET scans and positron emitting glucose to precisely determine when the cancer cells have inactivated the trichothecene and started cycling again, as described in U.S. Patent 6,342,520.

## **Inhibitory Activity**

The bulk of this application deals with inhibitory, and not cytotoxic doses. This is fairly notable departure from prior art. Prior art administers cytotoxic doses of chemotherapeutics for short periods of time. By contrast, in one embodiment of present invention, trichothecenes are used as long term sustained treatment regimens more akin to a treatment of chronic lifelong diseases like diabetes rather than prior arts chemotherapeutic regimens. This embodiment address unmet needs such as holding the cancer and related angiogenesis in check indefinitely, in cases where neoplasms in the lung are unresponsive to chemotherapy.

Another embodiment of present invention uses inhibitory doses administered during the time between administration cycles of prior art chemotherapeutics, to insure the integrity of the Skipper log cell kill model. The importance of this cannot be ignored in phase specific chemotherapeutic regimens, as applicant discloses in application 09/991,427 that prior art phase specific chemotherapeutic regimens fail to achieve high cure rates for cancer because of Gompertzian acceleration, or reduction in cancer cell cycle time, induced by the chemotherapeutic reduction of tumor size, which in turn results in the cancer's lack of phase synchronicity to subsequent administrations of the phase specific chemotherapeutic(s). The present invention discloses how to avoid this failure by using locally inhibitory doses of trichothecenes between chemotherapeutic administrations to halt the cancer cells from progressing through the cell cycle during the intervals between administrations of chemotherapeutics.

Another embodiment of present invention uses inhibitory doses for prophylactic purposes to prevent recurrence or metastasis of lung cancers. As such a brief discussion of the utility of trichothecenes as inhibitors follows.

*1) Inhibiting the Cell Cycle Control System* - Low doses of protein synthesis inhibitors stop actively cycling cells from cycling (without killing them). It has been shown that briefly inhibiting protein synthesis in early G1 (MBOC 896-897) stops cells from cycling by dismantling the cell cycle control system. After inactivation of the protein synthesis inhibitor, the cells require roughly

8 hours to rebuild their cell cycle control system and start cycling again.

A cycling cell is driven by an orderly sequence of events coordinated by the cell cycle control system. The cell cycle control system is dependent on hyperactive burst of protein synthesis of cyclins and cyclin dependent kinases (CDK). Fig. 3 shows the bursts of cyclin/CDK protein synthesis required to drive a cell through the various parts of the cell cycle. Fig. 4 shows the cell cycle control systems pathways involved in driving a cell through the G1 phase of the cell cycle. In a typical cell, inhibitory influences predominate and the normal state of a cell is not to cycle. In the non cycling cell, the Rb protein is bound to the E2F-DP1 transcription factor inhibiting it. When stimulated to proliferate, hyperactive protein synthesis of Cyclin/CDK is initiated, in particular Cyclin D/CDK4/6 complexes and Cyclin E/CDK2 complexes in G1, which phosphorylate the Rb protein, inactivating it, in turn activating the E2F-DP1 transcription factor. Inhibiting hyperactive protein synthesis of cyclins and CDKs prevents a cell from cycling. Cyclins such as cyclin D have short half lives (~ 15 minutes for cyclin D) and inhibiting their synthesis reduces their concentrations rapidly. After inactivation of the protein synthesis inhibitor, the cell requires 8 or so hours to rebuild its cyclin/CDK levels before it can resume cycling again.

*2) Inhibiting Overexpressed Oncogene Proteins* - Cancer happens through mutations that cause over expression or inappropriate expression of proteins responsible for growth (oncogenes) or mutations that disable genes responsible for inhibiting growth (tumor-suppressor genes). In cancers whose genetic profile includes overexpression of certain oncogene proteins, compositions of present invention would provide an additional mechanism of action by directly inhibiting the hyperactive synthesis of these oncogene products. Coincidentally, lung cancer happens to have such a profile. Approximately 25% of all lung cancers are Small Cell Lung Cancer (SCLC) and 75 % of lung cancers are Non-small Cell Lung Cancer (NSCLC). Studies have shown that lung cancer cells have acquired a fairly large number of genetic lesions (perhaps 10 or more) and the profile of oncogene related protein product is heavily dominated by protein overexpression (HPIM pg 554) and as summarized below:



Present in % of Cancer Cells Examined

<u>Oncogene Abnormalities</u>	<u>SCLC</u>	<u>NSCLC</u>
myc family overexpression	> 50 %	> 50 %
bcl-2 overexpression	> 75 %	> 50 %
Her-2/neu overexpression	< 10 %	~ 30 %
Telomerase overexpression	> 90 %	> 90 %
ras mutations	< 1 %	~ 30 %

Trichothecenes, as a preferential inhibitor of hyperactive protein synthesis, would also inhibit overexpression of growth factor proteins coded for by these oncogenes.

*3) Inhibition of Angiogenesis* - Cancer also involves another process that is dependent on hyperactive protein synthesis and hyperactive cell proliferation: angiogenesis. Angiogenesis is the growth of new blood vessels. Without angiogenesis a tumor cannot grow beyond the size of a pinhead (~ 1 to 2 cubic millimeters). The naturally occurring balance between stimulators and inhibitors of angiogenesis in the human body is also one in which inhibitory influences predominate. The rare instances in which angiogenesis occurs under normal conditions are wound healing, organ regeneration, embryonic development, and female reproductive processes. Also, when cells are deprived of oxygen or nutrients, they release angiogenic factors that induce new capillary growth which is why nearly all vertebrate cells are located within 50  $\mu\text{m}$  of a capillary. Unnatural or disease conditions that involve angiogenesis include cancer. As tumor cells crowd each other, crowded tumor cells become deprived of oxygen or nutrients, begin accelerated production of protein growth factors for angiogenesis such as VEGF, and issue these protein signals to the nearby endothelial cells (cells that form the wall of blood vessels). A normally dormant endothelial cell becomes activated, secretes enzymes that degrade the extracellular matrix (the surrounding tissue), invades the matrix, begins dividing and eventually the new endothelial cells organize into hollow tubes creating new networks of blood vessels. Endothelial cells can show a 100 fold increase in proliferation during neovascularization and it is known that tumors become vascularized at a size of less than 1250  $\mu\text{m}^2$ .

Trichothecene administration would thus function two ways to inhibit angiogenesis. First, it would dismantle the cell cycle control system in endothelial cells by the same cyclin/CDK mechanisms described for cancer cells. Second, it would inhibit the excessive production of protein growth factors such as VEGF by tumor cells that initiate angiogenesis. In addition to acting as a mitogen, VEGF has been shown in vitro to downregulate integrins (cellular adhesion molecules) which in turn should release the endothelial cell from density dependent inhibition of cell division (via downregulation of proteins such as P27 shown in Fig. 4). Furthermore, the VEGF activated endothelial cell itself begins to secrete enzymes to degrade the extracellular matrix before it starts to divide. Although all of this is part of the normal sequence of the endothelial growth process, loosening cell to cell and cell to matrix adhesion, would likely provide the perfect situation where relatively untethered cancer cells could slip between relatively untethered endothelial cells and enter the blood stream. Trichothecene would inhibit VEGF production, preventing downregulation of integrins and activation of endothelial cells, thus making it more difficult for cancer to enter into the bloodstream. Inhibiting angiogenesis not only starves the tumor of oxygen and nutrients but also reduces the probability of metastasis.

*4) Inhibition of MDR Proteins* - Inhibition also has applications in the phenomenon of multidrug resistance (MDR). MDR is characterized by massively amplified numbers of copies of a small segment of the genome which often contains a specific gene, known as the multidrug resistance (*mdr1*) gene. This gene codes for a plasma-membrane-bound transport protein ATPase. ATPases are a diverse family of transport proteins and overexpression of MDR protein in human cancer cells enables them to pump drugs out of the cells. As protein synthesis inhibitors, composition of present invention would inhibit overexpression of MDR proteins, impairing the ability of cancer cells to pump out the chemotherapeutic(s). This would involve a rationally designed transition from discontinuation of administration of therapeutics of present invention and administration of chemotherapeutics to take advantage of the MDR impaired state.

Inhibiting cancer and related angiogenesis, indefinitely, is a last hope for patients with neoplasms in the lungs that are inoperable and unresponsive to chemotherapy. A lifelong treatment regimen

would generally be preferable to death and particularly when it may be as simple as periodically smoking a cigarette containing trichothecene(s) .

The paradigm of present invention is also vastly different from prior art in that it is organ specific rather than cancer specific. Both inhibitory and cytotoxic dose levels of compositions of present invention focus on clearing out or immediately arresting the growth of neoplasms in the lungs, no matter where they came from (e.g. whether indigenous or metastasized from the breast, prostate, melanoma etc...). Methods of present invention do not focus on eliminating lung cancers that have metastasized to distant sites. Methods of present invention will however provide another tool in the growing arsenal of tools for use against cancer.

Lastly, inhibitory methods of present invention provide a means for preventing or reducing inflammation in the lungs as well as the severity of allergy attacks and allergic asthma attacks. Allergens cause the conversion of B-cells into plasma cells, which in turn generate large quantities of immunoglobulins. These cells are primarily in the bone marrow or blood. The target of these immunoglobulins are mast cells which are located in the lungs and would be subject to inhibitory influences of trichothecenes. Mast cells are activated by the immunoglobulins and initiate hyperactive protein synthesis for newly formed mediators of anaphylaxis including cytokines, leukotrienes, thromboxane, and platelet activating factor. Activated mast cells also release pre formed mediators of anaphylaxis including histamine, heparin, tryptase, kallikrein and chemotactic factors. Administration of inhibitory doses of trichothecenes well in advance of expected exposure to allergens would downregulate any of the pre formed substances, muting any allergic response. Administering inhibitory doses at initiation of an allergic response would inhibit hyperactive production of the newly formed substances.

Prior art drug therapies include various strategies (HPIM 1423 -1425) for either interfering with the allergic response pathway or counteracting its symptoms. These include adrenergic stimulants, methylxanthines, anticholinergics, glucocorticoids, and mast cell-stabilizing agents. The mast stabilizing agents in use today, cromolyn and nedocromil, work by preventing the release of the pre formed mediators of anaphylaxis. Compositions of present invention prevent synthesis of the

newly formed mediators by the mast cells. Thus the novel mechanism of action of present invention would very well complement the prior art, providing a complete solution: inhibition of release of pre formed mediators (prior art) and inhibition of newly formed mediators (present invention).

In cases such as COPD described below, CD8+ T lymphocytes and B lymphocytes comprise the primary inflammatory infiltrates in the lungs. If T or B lymphocytes are present in the lungs (other than in the blood), trichothecenes would provide a broad spectrum inflammatory inhibitor, interfering with multiple steps all along the inflammatory pathway, starting with inhibition of hyperactive production of effector proteins by T cells (e.g. IL-4, IL-13, IL-9), inhibition of hyperactive production of immunoglobulins (e.g. IgE) or other mediators by B cells, and inhibition of hyperactive synthesis of new mast cell product discussed above.

### **Toxic Activity**

At high doses, trichothecenes are toxic to all cells, including normal, non proliferative cells. The present invention proposes a truly novel curative, rather than just palliative, solution for COPD based, in part, on the ability to inflict a certain percentage of destruction to non proliferative lung tissue. In patent 6,355,251 applicant employs topical application of trichothecenes to skin to achieve a desired amount of epidermal chemexfoliation and in application 10/059,653 for a method of chemosurgery applicant employs interstitial administration of locally toxic doses of trichothecene directly into a tissue mass targeted for ablation. The chemical debridement of the lungs embodiment of present invention varies in several important respect from the other application and patent. First, the administration method of inhalation is novel over the topical application for skin or interstitial administration for tissue masses. Inhalation effectively deposits the trichothecene onto the mucus blanket in the lungs, which required disclosure of both the characteristics such as water insolubility that prevent elimination of the trichothecenes, as well as the in vivo models of both the Cleveland infants and AMRIID tests, that showed the trichothecenes were not prevented from internalizing in the lungs because of the mucus blanket and were not carried away into

systemic circulation by the cephalad movement of the mucus blanket. The mucus blanket effectively allows for dry inhalation of the trichothecenes as disclosed by applicant, versus the use of creams or solvents as required in the epidermal chemexfoliation or chemosurgery applications. Additionally, therapeutic benefit in epidermal chemexfoliation starts in the cytotoxic range as the lowest layers of the epidermis are rapidly proliferating tissue, versus the lungs which are not rapidly proliferating tissue and require toxic doses for therapeutic benefit. The claims of present invention relating to locally toxic doses are written narrowly as an inhalable chemical debridement of the lungs to avoid duplicating claims contained in above referenced patent and application.

There are ~ 16 million COPD patients in the United States. COPD is a group of chronic, slowly progressive, respiratory disorders and is made up of emphysema and chronic bronchitis. COPD is the fourth leading cause of death and the only one of the top 10 for which mortality rates are still rising. COPD results from persistent inflammation (particularly from smoking) and results in narrowing of both large and small airways. Airway epithelium is characterized by squamous metaplasia (abnormal transformation of epithelium into scaly cells), atrophy of ciliated cells, an hypertrophy of mucus glands ( increase in bulk). The remodeled epithelium actively produces cytokines that amplify and sustain the inflammatory process. Small airway transformation also includes overproduction of smooth muscle and goblet cells, peribronchial fibrosis, edema, intraluminal mucus plugs, and CD8+ T lymphocytes and B lymphocytes comprise the primary inflammatory infiltrates.

Palliative treatments include bronchodilators, glucocorticoids, and oxygen. Curative treatments are limited to lung transplants and Lung Volume Reduction Surgery (LVRS), an operation where severely emphysematous lung tissue is resected resulting in 25% to 50% improvement in airflow. LVRS mortality ranges from 5% to 18%, requires a hospital stay from 9 to 18 days, and costs \$ 33,000 to \$ 70,000 per case.

In this embodiment of present invention, trichothecenes are used in locally toxic doses to effectuate a respiratory tract chemical debridement treatment regimen for the lungs.

## **Reduction to Practice Examples**

Examples are provided to further give guidance on methods of use of compositions of present invention as discussed above. Satratoxin H is used in the examples because it is known to be highly efficacious by inhalation in its raw, dry powder form (see U. of Minnesota printout enclosed) and the representative macrocyclics follow an almost identical dose curve so Satratoxin G's was used for H, however any suitable trichothecene could be substituted in the examples.

### **Example 1: Use of Therapeutics as Stand Alone Treatment of Lung Cancer**

Lung cancer is the deadliest cancer resisting most conventional treatments. The average 5 year survival rate for all stages of lung cancer combined is currently only 14% . Chemotherapy today offers only “modest survival benefits (of 1 to 2 months)” and “complete clinical regression of tumor ... occurs in less than 5 % of cases” and as such use of prior art chemotherapy “requires careful judgment to balance potential benefits and toxicity” (HPIM p 560). Clearly a better solution is needed over prior art.

#### ***Example 1a: Inhibitory Treatment.***

A patient presents with inoperable lung cancer and is unresponsive to chemotherapy. Under prior art they are given several months to live.

Under present invention, they are administered a 2000 ng inhibitory dose (or any other suitable inhibitory dose), of satratoxin H (or any other suitable trichothecene) by dry powder inhaler (or any other suitable method of inhalation). This administration is repeated once every 10 days (or other suitable PET determined time period as previously disclosed) and administrations are continued indefinitely. Every few months the trichothecene administration may be suspended for 2 weeks (or other suitable time period), the drug vacation insuring any required normal cell cycling may take place, before once again resuming the inhibitory regimen.

The 2000 ng inhibitory dose represents roughly 60% protein synthesis inhibition, which prevents

cancer cells from cycling or developing related angiogenesis by preventing the hyperactive bursts of cyclin/CDK required to do so. The 60% attenuated protein synthesis does not affect the viability of normal, non proliferating tissue in the lungs.

*Example 1b: Cytotoxic Treatment.*

A patient presents with inoperable lung cancer and is unresponsive to chemotherapy. Under prior art they are given several months to live.

Under present invention, they are administered a 10,000 ng cytotoxic dose (or any other suitable cytotoxic or even partially toxic dose), of satratoxin H (or any other suitable trichothecene) by dry powder inhaler (or any other suitable method of inhalation). This administration is repeated by PET determined intervals, as previously disclosed (or other suitable time period), until no tumor is detected.

The cytotoxic dose levels provide multiple pathways of toxicity to the cancer cells over prior art's single pathway chemotherapy, as previously disclosed. Additionally, PSIs shut down the cancer's ability to produce MDR proteins, which is the primary reason for unresponsiveness of the tumor to prior art chemotherapeutics.

Example 2: Use of Therapeutics as Stand Alone Treatment of Breast Cancer in the Lung

Nearly half of patients treated for apparently localized breast cancer develop metastatic disease. Approximately 66% of breast metastasis involve the lungs.

*Example 2a: Inhibitory Treatment.*

Several months after treatment for breast cancer it is detected that the breast cancer has metastasized into the lungs. The patient is immediately administered a 5000 ng inhibitory dose (or any other suitable inhibitory dose), of satratoxin H (or any other suitable trichothecene) by dry powder inhaler (or any other suitable method of inhalation). This administration is repeated once every 10 days (or other suitable time period) until a determination is made on what chemotherapeutic, surgical, or other procedure will be undertaken to combat the metastasis.

Present invention provides a method of immediately arresting the cancer as well as providing prophylactic activity against metastasis (via VEGF/integrin downregulation previously disclosed).

*Example 2b: Cytotoxic Treatment.*

Several months after treatment for breast cancer it is detected that the breast cancer has metastasized into the lungs. The patient is administered a 10000 ng cytotoxic dose (or any other suitable cytotoxic dose), of satratoxin H (or any other suitable trichothecene) by dry powder inhaler (or any other suitable method of inhalation). This administration is repeated, as previously disclosed, by PET determined intervals (or other suitable time period) until no tumor is detected.

The example illustrates the point that present invention is organ specific (i.e. for neoplasms in the lungs) and not cancer specific as in prior art.

Example 3 : Method of Use in Conjunction With Conventional Chemotherapy - The chosen course of treatment for a patient with lung cancer is administration of paclitaxel and carboplatin, the leading cytotoxic chemotherapy for treatment of lung cancer.

*Example 3a: Insuring Integrity of Skipper Log Cell Kill Model / Preventing Chemotherapeutic Induced Gompertzian Acceleration.*

After the terminal half life of an administration of paclitaxel and carboplatin (or any other chemotherapeutic regimen), the patient is administered a 4000 ng inhibitory dose (or any other suitable inhibitory dose), of satratoxin H (or any other suitable trichothecene) by dry powder inhaler (or any other suitable method of inhalation). This administration is repeated as necessary to prevent any cancer cells from resuming cycling and said administration is discontinued not less than the trichothecene's inactivation time plus 8 hours (or other suitable time, preferably as determined by PET scan methods previously described) prior to the administration of the next cycle of paclitaxel and carboplatin, after which the trichothecene is once again administered in the same manner as described above.



The purpose of the inhibitory doses in this example is to prevent any growth in the cancer between chemotherapeutic administrations, as required by the Skipper log cell kill model. When used in conjunction with phase specific chemotherapeutics in this example, the purpose of the inhibitory dose is to prevent any progression of the cancer cells through the cell cycle (by dismantling the cyclin/CDK cell cycle control system) and preventing any Gompertzian acceleration of cancer growth rate (i.e. reduction in cancer cell cycle time) induced by chemotherapeutic reduction of tumor size, which in turn would make the cancer lose phase synchronicity with subsequent administrations of the phase specific chemotherapeutic, which in turn would result in failure to provide curative outcome.

*Example 3b: MDR Inhibition, Cancer Cytotoxicity, Regimen Integrity*

A lung neoplasm(s) is unresponsive to treatment with paclitaxel and carboplatin (or any other chemotherapeutic regimen). It is determined the tumor produces large amounts of MDR (multidrug resistance) proteins. The patient is administered a 10,000 ng cytotoxic dose (or any other suitable cytotoxic or mildly toxic dose), of satratoxin H (or any other suitable trichothecene) by dry powder inhaler (or any other suitable method of inhalation). In 10 days (or other suitable time period, preferably as determined by PET scan methods previously disclosed) paclitaxel and carboplatin (or any other chemotherapeutic regimen) are administered, in conjunction with or followed shortly thereafter (i.e. after the terminal half life of the prior art chemotherapeutics) by administration of another 10,000 ng cytotoxic dose of trichothecene(s). The prior art chemotherapy followed by 10,000 ng of trichothecene is repeated for the duration of the 6 or 8 cycles of prior art chemotherapeutic regimen as defined by the Skipper log cell kill model.

The purpose of the initial cytotoxic dose of trichothecene is not only to kill a percentage of the tumor, which allows for better subsequent circulatory transport of prior art chemotherapeutics, but to inhibit production of MDR proteins before administration of prior art chemotherapeutics, to prevent them from being pumped out by the cancer cells. The large doses also takes a long time to inactivate providing the inhibitory benefits between administrations of prior art chemotherapeutics as described in Example 3a. Lastly, the cytotoxic trichothecene dose boosts kill rate as an additional chemotherapeutic in the regimen, and it does so without any increase in systemic

toxicity because of its localization attributes, unlike the addition of prior art systemically toxic chemotherapeutics to a regimen.

#### Example 4: Prophylactic Activity for Patients at High Risk for Lung Cancer

A heavy lifelong smoker, that cannot or chooses not to quit smoking, is approaching 50 years of age, and is at high risk for contracting lung cancer and wants to insure a cancer will not start growing and metastasize before their next examination for lung cancer.

The patient is put on a continuous low inhibitory dose administration regimen, comprising smoking a cigarette(s) capable of delivering a sustained 1800 ng inhibitory dose (i.e. 50% protein synthesis inhibition) (or other suitable dose), of satratoxin H (or any other suitable trichothecene) with brief, periodic drug vacations to allow for normally required cell division for repair. The administration of therapeutic is discontinued roughly 2 weeks before the next lung cancer examination. After the examination by PET scan or any other suitable means is performed, the patient may go back on the prophylactic treatment regimen as described above.

The purpose of the inhibitory dose in this example is to prevent the hyperactive bursts of cyclin/CDK required to initiate or sustain cancer cell division or its related angiogenesis. Inhibition of VEGF prevents integrin downregulation and “leaky” blood vessels which facilitate metastasis.

#### Example 5: Inhaled Chemical Debridement Method for COPD

A lifelong smoker has severe emphysema and would like some degree of lung function restored however they cannot afford an LVRS or are scared to by the 18% mortality rate. Under present invention they would now have a chemexfoliation / chemical debridement option instead of surgery. The patient is administered a single normal tissue toxic level dose of 50,000 ng (or any other suitable toxic, but not lethal, dose necessary to kill a desired percentage of lung cells), of satratoxin H (or any other suitable trichothecene) by dry powder inhaler (or any other suitable method of inhalation).

In this example, the toxic doses are intended to eliminate a portion of the airway epithelium now

characterized by squamous metaplasia (abnormal transformation of epithelium into scaly cells), eliminate a portion of the increased bulk in the mucus glands, eliminate a portion of the remodeled epithelium that now actively produces cytokines that amplify and sustain the inflammatory process, and eliminate part of the overproduced smooth muscle cells and goblet cells.

#### Example 6: Inhaled, Localized, Suppression of Inflammation or Anaphylaxis

##### *Example 6a: Inhibition of COPD Related Inflammation.*

A COPD patient that has been a lifelong smoker is having trouble breathing because of ongoing inflammation in the lungs coupled with their destroyed cilia and virtually non existent cephalad movement, which is causing excessive fluid accumulation in the lungs. The patient is given a 3000 ng inhibitory dose (or any other suitable inhibitory dose), of satratoxin H (or any other suitable trichothecene) by dry powder inhaler (or any other suitable method of inhalation).

The purpose of the inhibitory dose in this example of COPD, where CD8+ T lymphocytes and B lymphocytes comprise the primary inflammatory infiltrates in the lungs, is to provide a broad spectrum inflammatory inhibitor, interfering with multiple steps all along the inflammatory pathway, starting with inhibition of hyperactive production of effector proteins by T cells (e.g. IL-4, IL-13, IL-9), inhibition of hyperactive production of immunoglobulins (e.g. IgE) or other mediators by B cells, and inhibition of hyperactive synthesis of mast cell product, as previously disclosed.

##### *Example 6b: Allergic Reaction or Allergic Asthma Prevention*

A person is going of a trip that will involve exposure to antigens known to trigger a severe allergic reaction or allergic asthma. The patient is given a 5000 ng inhibitory dose (or any other suitable inhibitory dose), of satratoxin H (or any other suitable trichothecene) by dry powder inhaler (or any other suitable method of inhalation) a day prior (or other suitable time period) to their expected contact with allergen. This administration may be used in conjunction with prior art drug treatments.

The purpose of the inhibitory dose in this example is to prevent activated mast cells from initiating hyperactive protein synthesis for newly formed mediators of anaphylaxis including cytokines, leukotrienes, thromboxane, and platelet activating factor. Since activated mast cells also release pre formed mediators of anaphylaxis including histamine, heparin, tryptase, kallikrein and chemotactic factors, administration of inhibitory doses of trichothecenes well in advance of expected exposure to allergens would downregulate reservoirs of any of these pre formed substances, muting any allergic response.

#### Other Examples, Applications and Embodiments

It should be noted that the above are only a few representative examples of numerous possible embodiments of present invention are nothing should be construed as limiting the scope of present invention to only the representative examples presented above. As an example, a COPD patient with lung cancer could be treated with mildly toxic doses (instead of cytotoxic doses ) as a their chemotherapeutic regimen. This would provide the additional benefit of chemical debridement to benefit the COPD as well as killing the cancer. As another example, a COPD patient that continues to smoke and does not want the COPD to progress, or wants to hold it in check after a chemical debridement procedure because they continue to smoke, would also benefit from continuous administration of inhibitory doses to prevent inflammation and proliferation of COPD related cells. As another example, cytotoxic doses could also be used to kill any currently dividing COPD related cells, which would not only prevent a new COPD related cell from being created but result in the death of the existing COPD related cell that started growing and dividing. These last three examples illustrate the point that a COPD patient could benefit from inhibitory, cytotoxic, and toxic doses depending on the circumstances and not just the toxic doses as more elaborately illustrated in the single representative Example 5.

Many other variants are also possible. The representative examples may include or exclude varying “drug vacation” periods for normal cell replacement to occur. The present invention also envisions the possibility of mixing the trichothecene with other compounds or substances, including combinations of trichothecenes, or substances that facilitate administration, facilitate or regulate the rate and/or depth of penetration and/or absorption of said trichothecene mycotoxins,

increase efficacy of said mycotoxins, facilitate retention of trichothecene in the lung by methods such as disabling cephalad movement of the mucus blanket, provide prophylactic activity against infection, or provide any other beneficial or synergistic function. The compounds collectively described above are termed herein “pharmaceutical compositions”. As an example, a combination of macrocyclic and simple trichothecenes may be used to achieve more extensive penetration (as macrocyclics internalize faster and simple trichothecenes would migrate further before internalization). As another example, antibiotics may be included as part of the “pharmaceutical composition”. As another example pharmaceutical compositions may include other protein synthesis inhibitors or angiogenesis inhibitors such as squalamine or troponin. As another example pharmaceutical composition may include any inert ingredients to facilitate or enhance distribution of therapeutics by inhalation. As another example, other agents that prevent inflammation or allergic response such as mast stabilizing agents cromolyn and nedocromil may also be included as part of the pharmaceutical composition. The examples provided in the application are only some of the potential uses of therapeutics of present invention and nothing in this application is intended to limit the potential uses of therapeutics of present invention for treatment of conditions of the lungs that could benefit from localized inhibition of protein synthesis.

The compositions of present invention cannot be administered to infants and young children. Care would also need to be taken that patients undergoing treatment with therapeutics of present invention do not inadvertently expose infants to therapeutics of present invention.

#### **References Cited:**

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